

How effective is preening against mobile ectoparasites?
An experimental test with pigeons and hippoboscid flies

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ABSTRACT

Birds combat ectoparasites with many defences but the first line of defence is grooming behaviour, which includes preening with the bill and scratching with the feet. Preening has been shown to be very effective against ectoparasites. However, most tests have been with feather lice, which are relatively slow moving. Less is known about the effectiveness of preening as a defence against more mobile and evasive ectoparasites such as hippoboscids. Hippoboscids, which feed on blood, have direct effects on the host such as anaemia, as well as indirect effects as vectors of pathogens. Hence, effective defence against hippoboscids is important. We used captive Rock Pigeons (*Columba livia*) to test whether preening behaviour helps to control pigeon flies (*Pseudolynchia canariensis*). We found that pigeons responded to fly infestation by preening twice as much as pigeons without flies. Preening birds killed twice as many flies over the course of our week-long experiment as birds with impaired preening; however, preening did not kill all of the flies. We also tested the role of the bill overhang, which is critical for effective preening against feather lice, by experimentally removing the overhang and re-measuring the effectiveness of preening against flies. Birds without overhangs were as effective at controlling flies as were birds with overhangs. Overall, we found that preening is effective against mobile hippoboscids, yet it does not eliminate them. We discuss the potential impact of preening on the transmission dynamics of blood parasites vectored by hippoboscids.

Keywords: Grooming; Behaviour; Defence; *Columba livia*; *Pseudolynchia canariensis*; Vector

1. Introduction

Birds are infested with a variety of ectoparasites including lice, mites, ticks, fleas and flies, all of which have the capacity to decrease host fitness (Atkinson et al., 2008; Møller et al., 2009). Birds combat ectoparasites with defences ranging from anti-parasite behaviour (Hart, 1992, 1997) to immune defences (Wikel, 1996; Owen et al., 2010). Grooming behaviour, which includes preening with the bill and scratching with the feet, is the first line of defence against ectoparasites (Clayton et al., 2010). Preening is an energetically expensive activity (Goldstein, 1988; Croll and McLaren, 1993); furthermore, the time and energy devoted to preening detracts from other behaviors such as feeding and vigilance (Redpath, 1988). Therefore, in order to be effective against ectoparasites while limiting its energetic cost, preening should be an inducible defence (Tollrian and Harvell, 1999). The importance of preening is illustrated by recent work demonstrating that features of bill morphology, such as the upper mandibular overhang, appear to have evolved specifically to enhance the effectiveness of preening for parasite control (Clayton and Walther, 2001; Clayton et al., 2005).

Nearly all of the work on the effectiveness of preening has been done with feather lice (Phthiraptera: Ischnocera), which are slow moving and therefore relatively easy targets for preening birds (Marshall, 1981; Atkinson et al., 2008). The effectiveness of preening for controlling more mobile ectoparasites such as fleas and hippoboscids flies has not, to our knowledge, been tested. Preening may also play a role in shaping vector ecology and the evolution of pathogens transmitted by ectoparasites.

The goal of our study was to test the effectiveness of preening against hippoboscids flies, which are mobile parasites of birds and mammals. Avian hippoboscids flies are dorso-ventrally flattened and very agile at slipping between the feathers. As described by Rothschild and Clay (1952): “They have... an extremely efficient method of moving among feathers - darting and scuttling about at a remarkable

speed - and are extremely difficult to catch on a living bird.” Hippoboscids may also be capable of avoiding preening by using “refugia” such as the vent region of the bird or behind the bases of the legs (JL Waite, personal observation).

Hippoboscid flies are a diverse group of parasites. More than 200 species are recognized, 75% of which parasitize birds belonging to 18 orders; the rest parasitize mammals (Lloyd, 2002; Lehane, 2005). Most species of bird flies are winged and capable of flight between individual hosts (Harbison et al., 2009; Harbison and Clayton, 2011). They spend most of their time on the body of the bird, where they feed on blood several times a day (Coatney, 1931). Hippoboscid feeding can cause anaemia (Jones, 1985), emaciation (Lloyd, 2002) and slow nestling development (Bishopp, 1929). Parents of hippoboscid-infested nestlings have lower reproductive success (Bize et al., 2004). Hippoboscid flies also transmit blood parasites that can have negative effects on birds, including malaria (Sol et al., 2003), trypanosomes (Baker, 1967) and possibly viruses such as West Nile (Farajollahi et al., 2005). In short, hippoboscids pose both direct and indirect threats to the health and fitness of their hosts.

To test the effectiveness of preening against hippoboscid flies, we used wild caught Rock Pigeons (*Columba livia*) that we experimentally infested with the pigeon fly *Pseudolynchia canariensis* (Diptera: Hippoboscidae). We conducted two separate experiments. The first experiment addressed two questions: i) do Rock Pigeons infested with flies increase the amount of time they spend preening and ii) is preening effective in killing flies? The second experiment addressed a third question: is the bill overhang important in the effectiveness of preening for fly control?

2. Materials and methods

2.1. Experiment 1: Preening and flies

Twenty-four Rock Pigeons were caught using walk-in traps in Salt Lake City, Utah, USA. The birds were transported to the University of Utah animal facility, where they were individually housed in wire mesh cages (30 x 30 x 56 cm) suspended over newspaper-lined trays. Each cage/tray was completely enclosed within a fly-proof net, which prevented flies from moving between birds in different cages. Birds were given ad libitum food, water and grit and kept on a 12-hour light/dark cycle. They were maintained in captivity for at least 6 months at low humidity prior to the experiment, which killed feather lice and their eggs that were present on the birds when they were captured (Harbison et al., 2008). Any flies present on pigeons when they were captured would have died during the 6 month period because the life span of pigeon flies is only 2-3 months (Fahmy et al., 1977). Since pigeons trapped in Salt Lake City do not usually have other ectoparasites, the birds were ectoparasite-free at the start of our experiment. Prior to the start of the experiment, birds were carefully examined to confirm that they did not, in fact, have any ectoparasites.

We blocked the 24 birds using two factors: i) location trapped and ii) time in captivity; we then randomly assigned birds to one of three treatments, with eight birds per treatment. All birds were sexed and weighed. Birds in the first two treatments were then infested with 20 flies each (10 male flies, 10 female flies), which is the maximum number recorded from wild pigeons (mean = 5.07 flies; Stekhoven et al., 1954). Flies used to infest birds were cultured from wild caught stock on pigeons kept for this purpose in another room. The third group of eight birds was not infested with flies.

Flies were removed from culture birds using CO₂ (Moyer et al., 2002). They were sexed under a microscope at 25x before putting them on experimental birds. Half of the birds (chosen at random) in each of the two fly-infested treatments had plastic attachments fitted to their bill to impair their ability to preen. The attachments are small C-shaped pieces of plastic that, when fitted in the nares of a

pigeon, create a 1.0 - 3.0 mm gap between the mandibles. This gap prevents the full occlusion of the bill needed for effective preening (Clayton et al., 2005). The attachments are harmless; they do not impair feeding or alter the amount of time that birds attempt to preen (Clayton and Tompkins, 1995; Koop et al., 2011).

To address our first question whether pigeons preen more when they are infested with flies, we compared the behaviour of birds with normal (unimpaired) preening with and without flies. Preening behaviour was quantified using instantaneous scan sampling between 13:00 and 16:00 hours (Altmann, 1974). Preening was defined as touching the plumage with the bill (Clayton and Cotgreave, 1994). Birds were observed at 6 s intervals (Clayton, 1990) for 30 observations per bird per day, for 5 days following infestation. We calculated the proportion of time that birds spent preening.

To address our second question whether preening is effective in killing flies, we compared the number of flies killed by birds with impaired preening to flies killed by birds with normal preening. The experiment lasted 1 week, after which one of the authors (JL Waite) removed dead flies from the bottom of each cage; food and water dishes were also checked for dead flies. Another author (AR Henry) re-examined all cages to ensure that nothing was overlooked. Damage to flies was observed and recorded under a microscope at 25x. Flies were scored as preening-damaged if the head, thorax, abdomen or at least one wing was crushed or missing, or if at least three legs were missing. We calculated the proportion of flies with preening-damage out of the total number of dead flies recovered for each host after 1 week.

2.2. Experiment 2: Bill overhang

Another 12 wild-caught (individually caged) pigeons were used for this experiment. Birds were again blocked by location trapped and time in captivity. Half of the birds, chosen at random, had their bill overhang trimmed away with a dremel tool. The other half was sham trimmed, i.e. they were

handled but no part of the bill was removed (Fig. 1). The trimming method, which is fully described in Clayton et al. (2005), does not harm the birds in any way. One week after trimming (or sham trimming) all birds were sexed and weighed, and then each bird was infested with 20 flies (10 males, 10 females). Preening behaviour and fly mortality were quantified as in Experiment 1.

2.3. Statistical analysis

Statistical analyses were performed in Prism[®] v. 5.0b (GraphPad Software, Inc.). Data were analyzed using Mann-Whitney U Tests for comparisons between two groups. ANOVAs were used for comparisons among three groups. The sex ratio of pigeon hosts in each experiment was compared using a Chi-square or Fisher's Exact test, as appropriate. Values are presented as mean \pm S.E. Results were considered significant at $P \leq 0.05$.

3. Results

Sex and body mass of hosts did not differ significantly by treatment in either experiment (Experiment 1: sex, Chi-square test, $P = 0.77$; mass, ANOVA, $F_{2,21} = 1.47$, $P = 0.25$; Experiment 2: sex, Fisher's Exact test, $P = 1.00$; mass, Mann-Whitney $U = 12.5$, $P = 0.42$).

3.1. Experiment 1: Preening and flies

Birds infested with flies preened more than twice as much as birds without flies; birds with flies preened $23.49 \pm 3.96\%$ of the time observed, whereas birds without flies preened $11.21 \pm 2.11\%$ of the time observed; (Fig. 2). The difference in preening rates between the two groups was statistically significant (Mann-Whitney $U = 10.5$, $P = 0.03$).

Birds with normal preening killed twice as many flies as birds with impaired preening; birds with normal preening killed $43.75 \pm 5.41\%$ of flies, compared with $21.88 \pm 5.74\%$ of flies killed by

birds with impaired preening (Fig. 3A). The difference in the number of flies killed was statistically significant ($U = 11.0, P = 0.03$).

Birds with normal preening also damaged a significantly greater proportion of dead flies than did birds with impaired preening (Fig. 3B; Mann-Whitney $U = 7.0, P = 0.01$). Of the dead flies recovered from normally preening birds, $44.6 \pm 0.06\%$ were damaged, while only $16.6 \pm 0.13\%$ of flies recovered from birds with impaired preening were damaged.

3.2. Experiment 2: Bill overhang

Removal of the bill overhang had no significant effect on preening time; birds without overhangs preened $12.96 \pm 1.08\%$ of the time observed, while birds with overhangs preened $16.81 \pm 3.90\%$ of the time observed (Mann-Whitney $U = 13.0, P = 0.47$). Birds with overhangs did not kill significantly more flies than birds with no overhang; birds with overhangs killed $50.83 \pm 11.93\%$ of flies, compared with $45.00 \pm 11.76\%$ of flies killed by birds with no overhang (Fig. 4; Mann-Whitney $U = 15.0, P = 0.69$). Thus, the bill overhang was not a factor in the efficiency with which preening killed flies.

4. Discussion

We examined the effectiveness of preening against mobile ectoparasitic flies. Pigeons experimentally infested with flies preened twice as much as pigeons without flies (Fig. 2). Preening also proved to be effective against flies (Fig. 3A); we recovered twice as many dead flies from the cages of birds that could preen, compared with those that could not preen. Pigeons were able to catch and crush flies (Fig. 3B), even though the flies are extremely adept at moving quickly and evasively through the feathers (Rothschild and Clay, 1952).

Removal of the bill overhang did not decrease the efficiency of preening significantly (Fig. 4). Clayton et al. (2005) showed that lice are crushed when birds preen by the mortar-and-pestle action of the tip of the lower mandible moving against the upper mandibular overhang. Although the overhang is essential for controlling feather lice, our results show that it is not needed when preening flies, presumably because the flies are much larger and softer-bodied than lice. Although preening proved to be an effective defence against flies, it did not eliminate all of them over the course of our week-long experiment. Only one of 40 birds in the two experiments cleared itself completely of flies.

Preening may have the added benefit of helping to protect birds from pathogens for which the flies are vectors. In principle, preening can prevent transmission of pathogens if it kills infected vectors before they have an opportunity to bite the host. The fly *P. canariensis* is a known vector of the blood parasites *Haemoproteus columbae* and *Trypanosoma hanna* (Fahmy et al., 1977; Mandal, 1991). JL Waite (unpublished data) recently showed that pigeons exposed to just five flies for 3 days can become infected with *H. columbae*. In our study, only an average of 50% of flies placed on pigeons were killed during the week-long experiment (Fig. 3A). Thus, even birds with relatively efficient preening may remain at risk of acquiring blood parasites. If preening irritates flies, encouraging them to move between hosts, then preening might even have the effect of increasing pathogen transmission (Hodgson et al., 2001). It would be very interesting to measure the impact of preening on pathogen transmission by hippoboscids among birds in a population.

We found that pigeons infested with flies doubled the amount of time that they spent preening compared with controls (without flies) and compared with the typical rates of preening for other pigeons and doves (Clayton, 1990; Koop et al., 2011). One might predict that experimental birds would spend even more time preening, given that they did not completely remove their infestations in most cases. However, research on the cost of preening shows that it is energetically expensive. When

birds preen, their metabolic rates increase by as much as 200% (Wooley, 1978; Croll and McLaren, 1993). The energetic cost of preening might explain why preening is an inducible defence against hippoboscids. Additional indirect costs of preening include the time taken away from courtship behaviour, foraging and predator surveillance (Redpath, 1988). Thus, in addition to the direct impact of hippoboscids on host fitness, flies may have indirect effects mediated by the energetic and time related costs of preening. Indeed, there may well be a trade-off between the indirect cost of preening and the more direct costs of fly infestation.

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Figure legends

Fig. 1. Rock Pigeon bill showing upper mandibular overhang before (A) and after (B) removal of the overhang. The overhang grows back after several weeks. Figure reproduced from Clayton et al. (2005).

Fig. 2. Proportion of time that birds with and without flies spent preening.

Fig. 3. Effect of preening and an example of preening damage. A) Proportion of flies killed by birds with normal versus impaired preening. B) Example of intact versus preening-damaged flies.

Fig. 4. Proportion of flies that were dead in cages of birds with and without bill overhangs.

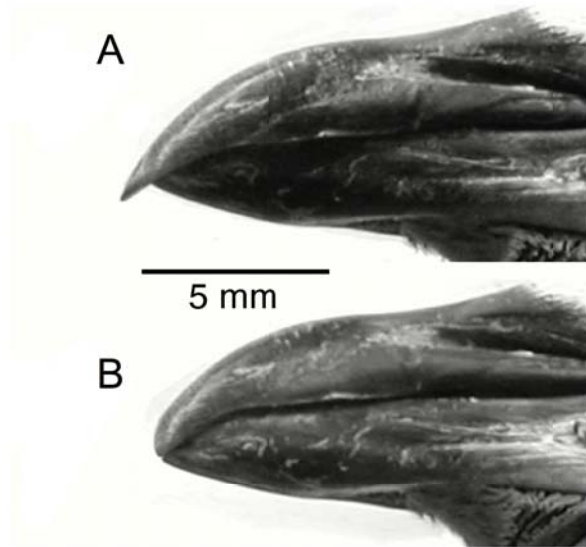


Fig. 1

Fig. 2

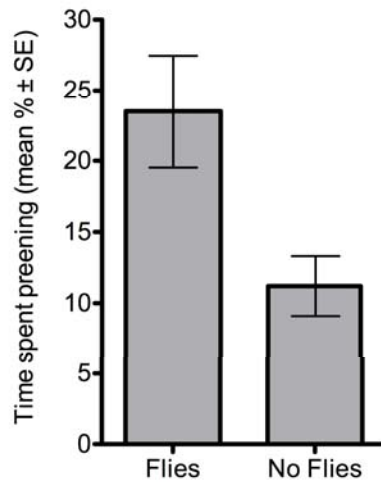


Fig. 3

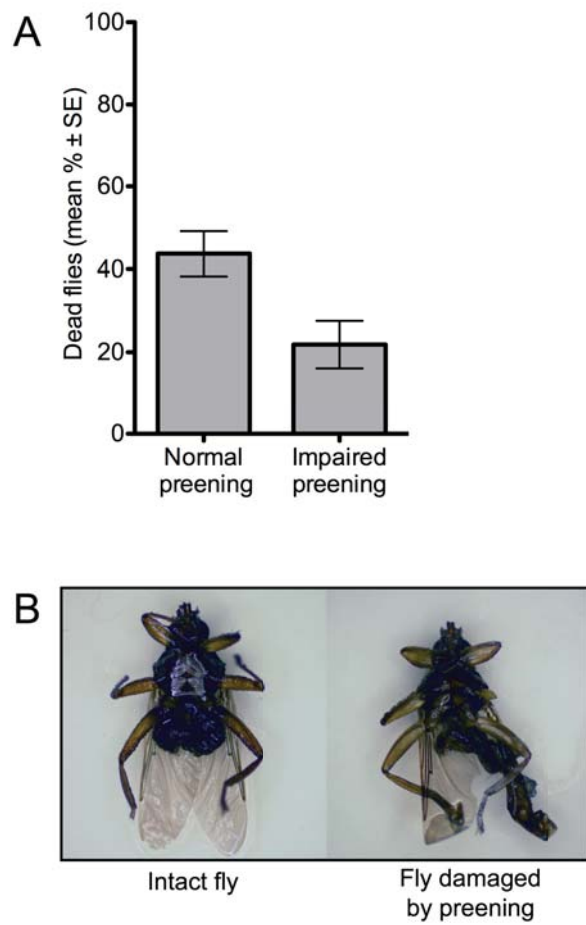


Fig. 4

